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Shelf-Life of Food Products in Modified Atmoshpere-Demonstration of A Packaging Equipment for Agricultural & Food Materials

Funded by
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ADF-DEMONSTRATION PROJECT ON

Shelf-life of Food Products in Modified Atmosphere-Demonstration of A Packaging Equipment for Agricultural & Food Materials

A Report Submitted to ADF

By

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ADF-DEMONSTRATION PROJECT ON

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1.0 Introduction

Loss in quality and shelf-life are the major problems in marketing of fresh plant and animal products. In many instances, especially in developing countries, these losses exceed 50%. Methods to reduce losses include preservation by heat, removal of water by dehydration, cold storage and freezing as well as chemical preservation or low dose irradiation. However, many of these methods are energy intensive and may change the texture, flavor and overall character of the food. Hence, from consumer's points of view, these conventional methods have some disadvantages. Moreover, there has been an increased consumer demand for high quality fresh products or fresh-like products.

Modified atmosphere packaging (MAP) is one method of preserving food products which provides fresher, nutritious, lower calorie, more convenient and economical fresh food products along with retaining the natural qualities and appearance of the product. The MAP technology can also be used as tool to effectively solve the problem of high costs associated with the inefficiencies of handling bulk fresh products.

Modified Atmosphere Packaging (MAP) can be broadly defined as any process which changes the environment around a product. Vacuum packaging is one example. Flushing a package with a gas or gas mixture is another. The package can also be evacuated and the atmosphere replaced with a predetermined, controlled gas or gas mixture. Thus, simply stated, MAP is a process by which the shelf-life of a fresh product is increased significantly by enclosing it in an atmosphere which slows down the degradative processes such as the growth of microbial organism, whilst enhancing some beneficial actions such as retaining the desirable red color of meat.

The term controlled atmosphere packaging (CAP) is also sometimes used. In both controlled and modified atmospheres the atmospheric composition surrounding the product is different from that of normal air. Most commonly the changes involve reduced oxygen and increased carbon dioxide concentrations. However, in some cases some other gases are also used. MA differs from CA in the precision of controlling the gas partial pressure. CA is usually used for long term storage (i.e. several months), while MA is applied in the transportation and marketing chain for short-term storage.The availability of packaging films with a wide range of physical characteristics and versatile packaging equipment has enabled MAP of food in small, convenient retail or distribution units and has opened unlimited options for application of this technique in food preservation.

Considerable fundamental knowledge has been published on the mechanism involved in the suppression of degradative reactions in the fresh commodities under low O_2 and elevated CO_2 levels in micro atmospheres. However, much more basic knowledge on chemical, physical and micro structural changes in stored commodities under process stress conditions is needed to support technological advances in MAP, mainly because the design of effective MA package system is dependent on the chemical and physical properties of the package components as well as information on the gas dynamics in the commodity containing package systems. Unfortunately, detailed data on the physical properties of the package components are not available. The on going commercial success in MAP technology for meat products and fruits and vegetables will rely on commercial availability of new functional type of barrier and permeable polymeric films. The design of suitable packaging systems to cope with temperature abuse situations is also required.

In the present report, the use of modified atmosphere packaging for the extension of shelf life of perishable products especially the fresh produce i. e. fruits and vegetables and meat, poultry and their products has been reviewed, with a view to identify the research gap in this important area of food preservation.

2.0 Review of Literature on MAP of Meat, poultry and their products

The storage life of meat is affected by initial microbial load, temperature of storage, the gas composition of MA, and gas permeability of the packaging materials.

2.1 Microbiological status of packed meat

The level of hygiene is a critical control point influencing safety and storage life of the product. High initial microbial contamination of meats negates the benefits of MAP [13]. The effect of initial microbial load on time to spoilage was illustrated by Kraft [41]. Under aerobic conditions, where spoilage is assumed at 10^7 colony forming units (CFU)/cm^2, an initial microbial load of 10^7 as opposed to 10^5 CFU/cm^2 has the potential to double the time of spoilage at a specified storage temperature. Under anaerobic conditions, where spoilage is not directly correlated with microbial counts, the time to reach maximum population would be similarly extended but the time of spoilage is more difficult to estimate. Depending on the type of organisms present, off-odors or off-flavors develop 2 to 3 weeks after the maximum population of 10^8 CFU/cm^2 is reached [16]. This depends in part on the composition of the microbial flora and the number of microbes initially present that are capable of growth in the MA [47]. The effect of high initial microbial load on storage life of packaged meats have been reported by several researchers [13].

2.2 Storage temperature

The temperature has been cited as the most important factor determining the storage life of MAP meats [53]. Numerous studies have demonstrated the effect of storage temperature on storage life. Storage of vacuum packaged beef cuts at 0°C consistently enhanced storage life compared with 5.5°C [12]. Increasing the storage temperature from 5 to 10°C in an atmosphere of 20% CO_2 in air caused a decrease in storage life from 11 to 6 days [9].

Elevation of storage temperature reduces the color stability of MAP fresh meat. At temperature above 3°C myoglobin is more readily oxidized to metmyoglobin. The

rate of discoloration of vacuum and nitrogen-packaged beef increases as storage temperature is increased from -1 to 5°C. The amount of purge or drip loss from MAP is also related to storage temperature. At temperature close to 0°C the water holding capacity of meat is maintained owing to reduced denaturation of protein.

2.3 Gas atmosphere

The starting point of MAP was vacuum packaging. . The gases used for MAP are CO_2, N_2, and O_2, separately or in combination. Carbon mono-oxide has been considered for application in MAP of red meats because of its potential to overcome discoloration [54]. Unfortunately, the color retention with CO can mask microbial spoilage. Moreover, Carbon mono-oxide is highly toxic because of its affinity for haemo- and myoglobin. Hence, its use requires special precaution. The role of CO in extending the storage life of red meat, however, appears to be only the stabilizing of meat color.

2.3.1 carbon dioxide

The CO_2 gas is used in the MA storage of meats for its well-documented bacteriostatic effect [9,10,11,32, 45]. Generally, CO_2 is used in combination with N_2 as an inert filler gas and (or) oxygen, although some workers recommend the use of 100% CO_2 [30]. The overall effect of CO_2 is to extend the lag phase and to increase the generation time of sensitive microorganisms. The efficacy of CO_2 in inhibiting microbial growth depends on (1) the sensitivity of meat spoilage organisms; (2) the concentration of CO_2; (3) storage temperature; and (4) the growth phase of microbial population. The inhibitory effect of CO_2 is selective. Molds and aerobic bacteria such as pseudo monads are inhibited, but it has only a limited effect on yeast and anaerobes such as lactic acid bacteria [45].

Decreasing the storage temperature increases the solubility of CO_2 and hence its bacteriostatic effect [9]. When CO_2 is added to the meat atmosphere, it is absorbed by the fat and lean tissue, resulting in decrease in pH due to the formation of carbonic acid [14]. However, CO_2 inhibition of microbial growth occurs in buffered media

[40]. The inhibitory effect of CO_2 has not been fully elucidated but it may be related to interference with enzymatic activity in some species [16]. Other theories on mechanism of action of CO_2 include interference in membrane fluidity [53] and toxicity of undissociated carbonic acid.

It is generally agreed that for MA of fresh meat is 20 to 30%. Most of the studies on the effects of different CO_2 levels on the MA of beef were done in atmospheres containing O_2 concentration less than those present in air. Systems have been patented with use of O_2 levels of 50 to 80% and the balance of the MA filled with CO_2 [58]. These systems prolong the bright red color of chilled beef for atleast one week. In the absence of O_2, beef stored with elevated CO_2 does not discolor. The purple red color of deoxymyoglobin "blooms" to the cherry red color of oxymyoglobin when the meat is exposed to air [49]. There is some evidence of a residual bacteriostatic effect when meat stored in CO_2 is exposed to air [19]; however, the CO_2 must be present continuously for the on going inhibition of sensitive microorganism [11].

2.3.2 nitrogen

This inert gas is commonly used as a filler gas for MA. Originally it was thought that the anaerobic environment created by N_2 would extend the storage life of meat. However, packaging beef in 100% N_2 resulted in greater discoloration of meat compared with CO_2, O_2 or a gas mixture of 70% N_2, 25% CO_2, and 5% O_2 [32]. In contrast, packaging in 100% N_2 caused less discoloration than vacuum packaging for beef [57]. Nitrogen reduces purge as a result of increased water-holding capacity [54,57]; however, it has little or no bacteriostatic effect [54].

2.3.3 oxygen

When O_2 is used in high concentration (greater than 50%) it has two possible functions : (1) to maintain the bright red color of meats [4], and (2) to control the growth of some spoilage organisms. A gas atmosphere containing 70-80% O_2 and 15% CO_2 extended the odor and color storage life of MAP beef at 5°C for upto 9 d compared

with storage in air [10]. In contrast, other researchers have reported reduced storage life due to metmyoglobin formation and oxidative rancidity [48,54]. These conflicting results may reflect differences in meat type; for example lamb chops stored in an oxygen- enriched atmosphere developed rancid flavor in 3 weeks [48], whereas lipid oxidation was not limiting factor in oxygen enriched storage of pork [3]. Low concentration of oxygen in MAP (less than 10%), for example 2% O_2, 20% CO_2 and 78% N_2, extended the storage life of beef compared with packaging in air, but vacuum packaging and an MA of 100% CO_2 were most effective [21]. Gas mixture containing 10% O_2 did not maintain the color of beef stored at 4°C [4].

2.4 Gas permeability & Packaging systems for meat

Gas permeability of packaging materials determines the rate of change of the gases in MAP. For retail cuts of meat, where retention of bright red color is desired, packages with high oxygen transmission rate are used. However, for cuts of meats, where extended storage life is the primary concern, packages with low gas transmission rates are used [47,58]. When assessing information on the efficacy of MAP meat, the stage of development of gas-impermeable packages must be taken into account. Foil laminates can exclude virtually all exchange of gases. Plastic films have now been developed that can achieve this. Extension of storage life beyond 6 weeks is generally achieved with storage temperatures of 0 to -2°C, excellent hygiene of carcass meats, 100% CO_2 and gas impermeable barrier film [29]. Storage life of vacuum packaged beef, measured by discoloration and off-odors is inversely related to the gas (oxygen) permeability of the wrapping film [47,54]. Various systems of over-wrapping have been developed for red meats, for example, the retail cut is packaged in a gas permeable film and overwrapped in an anaerobic MA containing elevated levels of CO_2. When the overwrap is removed, oxygen passes the gas permeable film and oxygenates the myoglobin. Vacuum packaging of retail meats is best adapted to processed meats, but application of vacuum- or MAP techniques to fresh meat for retail sale is also an obvious attraction. Centralized packaging

of retail meats would reduce the amount of plastic material used for marketing of fresh meats.

Gas permeability of the packaging film depends on the material used. Both polyvinylidene chloride (PVDC) and ethylene vinyl alcohol (EVOH) have excellent water vapor and gas barrier characteristics. Meat packaging films are generally multi-layer structures using a variety of polymer resins [28]. For prolonged refrigerated storage of fresh and processed meats a low O_2 transmission rate is required. The O_2 transmission rate is a function of polymer type, thickness, storage temperature, relative humidity, and other factors. Until recently (the end of the 1980s), clear plastics couldn't achieve O_2 transmission rates of 25-30 $mL/m^2/24$ h at 23°C and 0% RH. However, lower O_2 transmission rates have been achieved with new clear, composite polymers with O_2 transmission rates < 1 $mL/m^2/24$ h. Sealing of MA package is a critical step in forming and maintaining the desired atmosphere in the package.

2.5 Conclusions & Research needs for meat products

Vacuum or MA packaging of meats with elevated CO_2 has revolutionized meat marketing. Many research studies have focused on the effect of gas atmosphere on extension of storage time. It appears that a minimum of 20% of CO_2 is necessary for the inhibitory effect, but some reports indicates that 100% of CO_2 is optimum [29]. There may be little or no economic advantage to differ amount of CO_2, hence the optimum concentration of CO_2 should be determined under a wide range of storage conditions. The microbiology of anaerobic MAP meats has been extensively studied and extensive studies have been done on processed meats to determine their safety with temperature abuse.

It is well known that the bright red color of fresh meats is an important criterion of meat quality particularly from consumer's acceptance point of view. Several studies have been conducted but processing conditions to optimize the suitable gas combination to retain this desired color could not be achieved. Hence there seems to be need to study the diffusion characteristics of different gases in the meat to maintain the

bright red color. Such study [20] was carried out Enfors and Molin (1984) in which they measured the CO_2 evolution in pork loins stored in air or nitrogen at $5^{o}C$. In a recent study [2] it was found that interactive packaging using oxygen absorbers with concomitant development carbon dioxide and packaging material with low oxygen transmission rate (OTR) completely eliminated discoloration of pasteurized, sliced ham normally encountered as a result of photo-oxidation. They also found this procedure to be superior to conventional vacuum packaging with regard to overall sensory evaluation and microbial load. Another important aspect which need attention is the textural properties and changes that occur in them when packed in combination of different gases and stored at different conditions. A study [15] on effect of processing conditions and additives on the texture of stored freeze-dried beef were conducted. However, such studies on meat products packaged with MAP are lacking. Hence this aspect also need to be studied.

3.0 Modified Atmosphere Packaging of Fruits and Vegetables

The effects of reduced O_2 and elevated CO_2 in extending storage life in fruits and vegetables have been demonstrated in research and commercial applications of controlled atmosphere (CA) storage [33]. Besides reduced respiration, these benefits arise through reduced ethylene (C_2H_4) synthesis and activity. This retards ripening in fruits and toughening in some vegetables [7]. Elevated CO_2 with or without reduced O_2 has been shown to reduce chlorophyll breakdown [56]. In cut produce, low levels of O_2 retard enzymatic browning at cut surfaces. While CO_2 levels may not be high enough to directly retard microbial spoilage [23] this can be directly slowed by the improved condition of the stored produced [5].

Hence the benefits of MAP of fruits and vegetables are obvious. To study the consumer perception of MAP products. a study by Agriculture Canada [1] was initiated. The results of the study implied that a significant segment of Canadian population would pay premium prices for MAP products, particularly fresh produce and single-serving products. Some MAP opportunity targets for horticultural products were identified as lettuce, strawberries, peaches, cauliflower, mushrooms, blueberries, raspberries, broccoli and pears.

3.1 Diffusion of Gases within fruits and Vegetables under MAP

Katan [38] studied the role of diffusion in food packaging. The dynamic state 0f the gases within the package system is due to the on-going utilization of O_2 in the respiration pathways for the oxidation of hexoses and other energy sources and to the evolution of respiratory CO_2. During the respiration of MAP commodity, concentration gradient are established between the gases in the tissue and the microatmosphere gases and outside air. With a depleting O_2 environment in the tissue, O_2 is transferred from the outside air through the packaging material into the microatmosphere and finally into the tissue. The rate of O_2 transfer to the cells will depend on the partial pressure differences

along the path, the diffusion coefficient of the packaging material, resistance of gas diffusion in the tissue, and the temperatures of the environmental air, the microatmosphere, the commodity and the package system. The accumulating respiratory CO_2 in the tissue will travel in the opposite direction with the rate of transfer to the outside air being governed by the same factor mentioned above for O_2 transfer rate. Eventually, a gas equilibrium is established in the microatmosphere with specific levels of O_2 and CO_2 [31]. Many researchers have developed predictive equations for selecting polymeric films with gas permeabilities required to achieve O_2 and CO_2 levels in the microatmosphere for MA packages [31,37].

As the partial pressure of O_2 ($p\,O_2$) declines in the microatmosphere, a level is reached in a fruit or vegetable when insufficient O_2 is available for maximum rate of respiration. As the

$p\,O_2$ is reduced further, the respiration rate declines as a function of $p\,O_2$. The reduction of the respiration rate may be attributed to the suppression of low O_2 affinity enzymes such as ascorbic acid oxidase and glycolic acid oxidase [8]. At O_2 levels between 1% ($p\,O_2$=0.01) and 7% ($p\,O_2$=0.07 ATM) in the microatmosphere of a commodity, the respiration rate is approximately halved [6,61]. Resistance (r) of gas diffusion through the boundary gas layer and skin of most fruits and vegetables with pores varies from 5000 to 50000 s/cm with O_2, CO_2 and ethylene [6]. After passage through the skin, O_2 is transported through interconnected intercellular gas spaces (micro conduits) by diffusion within an O_2 gradient between just under the skin and commodity center [6,37].

3.2 Responses of fruits and vegetables to low O2 and high CO2 levels in modified atmospheres

. In MAP the reduced O2 and elevated CO2 levels can be beneficial by decreasing the respiration rate, ripening process, ethylene synthesis rate, ethylene sensitivity to ripening and tissue softening [36,37,61]. Kadar [34] has assigned

recommended MA levels of O_2 and CO_2 for the maintenance of quality attributes of a variety of fruits and vegetables.

Kadar and Morris [36] have assembled data on the relative tolerance limits of the fruits and vegetables to reduced O_2 and elevated CO_2 levels in MA. These tolerance limits of O_2 and CO_2 for a variety of fruits and vegetables. When O_2 level drops below the O_2 tolerance value for a commodity, specific physiological injuries and quality deterioration may occur. They further pointed out that the tolerance limits can vary as a function of type of commodity, cultivar, storage temperature, physiological age, storage time and added supplemental gases. Low temperature as an adjunct to MA is not always beneficial for vegetables. It has been found that lettuce stored at $0^{o}C$ under MA attained brown stain whereas at a storage temperature of $10^{o}C$, no brown stain was evident.

Below O_2 levels of 1 to 2% in the microatmosphere of a commodity, the aerobic respiration shifts over to anaerobic respiration which involves only glycolysis and the decarboxylation of pyruvic acid to acetaldehyde, which is then converted to ethanol. Anaerobic volatile compounds are formed in some fruits and vegetables to the extent that the commodities are no longer acceptable due to intense off-flavor [37].

3.3 Respiration in fruits & vegetables in modified atmospheres

Beneficial respiratory responses of fruits and vegetables to lowering the O_2 levels donot take place until the level reaches about 12% or less [37]. For apples and broccoli, the respiration rates are reduced by about one-half when the O_2 levels are dropped to about 4 to 5% [61]. Robinson *et. al.* [50] found that the respiratory response of 30 fruits and vegetables to 3% O_2 at 0, 10, $20^{o}C$ varied widely. The reduction of respiration rate in a 3% O_2 microatmosphere at $0^{o}C$ in comparison to an air (21% O_2) microatmosphere ranged from 10 to 46%, whereas at 10 and $20^{o}C$, the reduction was between 20 and 60%. When whole fruits and vegetables are sliced, the respiration rate increases and the heat production rises [51]. Rosen and Kadar [51] found that lowering the O_2 in the microatmosphere to 2% or below reduced the evolution rate of CO_2 by pear slices at $2.5^{o}C$

over a 7-day period. The respiration rates of some of the representative fruits and vegetables at different temperatures and in different environments have been reported by Kadar *et. al.* [37].

3.4 Ethylene production

When fruits and vegetables are exposed to microatmospheres with levels less than 8% O_2, the ethylene production decreases and the sensitivity of commodities to ethylene is reduced [7,59]. At an O_2 level of 2.5%, ethylene production was reduced to one-half the rate in comparison with air. Burg and Burg [7] observed that at a 3% O_2 level in the microatmosphere, ethylene binding was reduced to around 50% of that in air.

When the CO_2 level in fruit is elevated, the rate of ethylene synthesis may be reduced or promoted or may have no effect, depending on the CO_2 content and the commodity type [59]. In some commodities, the increase in ethylene production rate occurs only when the CO_2 is sufficiently high to cause physiological injury [35].

3.5 Effect of MAP on quality attributes

Quality attributes such as taste, odor, texture and color which are dependent on the chemical composition and structural features of fruits and vegetables may be retained by low O_2 and high CO_2 microatmospheres in MAP.

3.5.1 Flavor

During storage of fruits and vegetables, sugar may be converted into starch (peas, sweet corn) and bitter components (isocoumarin) may be synthesized under ethylene inducement. Thus, the taste profile of a fruit or vegetable changes with time storage, the rate being dependent on the temperature and the MA. Goodenough [25] reported that the tomatoes at 12.5°C in an MA of 5% O_2 and 5% CO_2 had an increase in glucose, fructose and citric acid levels, and a decrease in starch and malice acid content. Off-flavors in fruits and vegetables can be produced by subjecting them to low O_2 (lower than 2%) and high levels of CO_2 (15% and above). Anaerobic off-flavor volatile may be decomposed by

submitting the produce to air storage. When broccoli is held under MA at 0.5% or less O_2 and more than 15% CO_2, a mercaptan/H_2S odor is evident in a few days at 5°C.

3.5.2 Color

The degradation of pigments in commodities can be reduced considerably by MA [33,60]. According to Singh *et. al.* [56], chlorophyll loss can be retarded in crisped lettuce by reduced O2 (2 to 5%) and elevated CO_2 (2.5%) levels in the microatmosphere. With elevated CO_2 contents in microatmospheres, pigment in green beans, apricots and peaches have been reduced [60]. Knee [39] found that the rate of chlorophyll degradation in the peel of apples was reduced by one-half by MA with 2.5 to 4% O_2 levels. Goodenough and Thomas [26], upon storage of tomatoes at 12°C in 2.5 to 4% O_2 and 4% CO_2 microatmosphere, found that the Chlorophyll degradation and lycopene synthesis were reduced.

3.5.3 Textural characteristics

The storage of harvested commodities under MA conditions can inhibit the synthesis of polygalacturonases and thus reduce the softening of the tissue [27]. Knee [39] reported that rate of tissue softening of apples was reduced by one-half when exposed to 2.5 to 4% O_2 levels.

Sharma *et. al.* [55] studied the kinetic analysis of post harvest texture changes in asparagus. In a recent study, Everson *et. al.* [22] studied the effects of modified atmospheres on textural and cell wall changes of asparagus during shelf-life. They found that rate of respiration and the extent of pack atmosphere modification were similar for each harvest. In non-perforated packs, CO_2 increased to >11% and O_2 fell to <2% and in the microperforated packs, CO_2 equilibrated to ca 5-6% and O_2 decreased to a minimum of ca 6-7%. The shear force needed to cut stem increased during 6 day shelf-life. Gariepy *et.al.* [24] studied the effect of precooling and modified atmosphere storage of green asparagus. They found that precooling the asparagus prior to storage reduced losses by 20% and the MA stored asparagus had better color, fresher appearance, firmer texture and lower mass

loss (< 12%) than that stored for same period under normal atmosphere with 80% RH (mass loss of 60%) or with >95%RH (mass loss of 41%).

3.6 Simulation studies on MAP of fruits and vegetables

Some simulation studies were conducted and mathematical models have been developed on post harvest changes in fruits and vegetable. Labuza *et. al.* [42] developed model for optimization of flexible film packaging of foods for storage while Saguy and Karel [52] developed model on quality deterioration during food processing and storage. Henig and Gilbert [31] developed computer-aided solution to the mathematical equations representing the changes in respiratory gas concentrations within tomato packages. The computer solution was then validated by comparing it to model package's results, and was further used to analyze the different packaging system variable's effect on internal package atmosphere.

The simulation studies specifically on MAP of fruits and vegetables are very limited. Mannapperuma *et. al.* [46] developed a mathematical model based on simultaneous gas diffusion and chemical reaction to represent the gas exchange in foods stored in modified atmospheres. The respiration process was developed as first order chemical reaction and gas transfer process was modeled as ordinary diffusion. Emond *et. al.* [17] conducted laboratory experiments to determine gas exchanges through perforation. They also developed empirical equations to predict the effective permeabilities to CO_2 and O_2 for different values of diameter, thickness and temperatures. A computer program was developed to simulate gas exchange for three kinds of package covering. Simulation results provided answers to the appropriate package for storage of broccoli and strawberries under modified atmosphere. Lee [44] developed a theoretical method to design a package to maintain a desired concentration of O_2 and CO_2 inside a single consumer package of fresh produce.

3.7. Conclusions & Research needs for MAP of Fruits & Vegetables

It is evident that significant quantum of fundamental knowledge has been published on the technique of modifying the atmosphere to enhance the shelf-life of perishables during storage. However, limited studies appear to have been attempted to fully understand the mechanism of gas diffusion in the packaged product. Additional information is needed on the resistance of fruits and vegetables to diffusion of O_2, CO_2 and C_2H_4 under different atmospheric and temperature conditions. Moreover, changes in Respiration Quotient (RQ) in response to changing atmospheres could have great evolving atmospheres inside packages. Hence, it is necessary to study this phenomenon specially for vegetables and like products. Another aspect which need to be studied is the textural properties and changes in them during storage. Data on many fruits and vegetables on these aspects is lacking and therefore need to be generated.

More sophisticated mathematical models for predicting packaging requirements for different commodities also need to be studied. This will be based on increased information in areas such as product requirements, the effects of MAs on respiration rate, and the effects of temperature on the package permeability. Most data on packaging film permeability are generated at single temperature and often at very low relative humidity. The responses of film permeability to temperatures between 0 and 20°C to relative humidities between 85 and 95% must be determined. If possible, irradiation may be combined with MAP as indicated in some of the recent studies. Radiation and MAP of strawberries extended the shelf life by 50-100% [43].

4.0 Equipment & Accessories

4.1 Audion/pac vacuum chamber machine (Model-VM151)

The machine was purchased to conduct experiments on modified atmosphere packaging in a laboratory scale. The machine is equipped with a high vacuum/high capacity pump for efficient packaging and is microprocessor controlled with complete programming of each step of cycle viz. vacuuming, gas flushing and sealing. This provides great flexibility in use of various packaging materials and different levels of vacuuming and sealing timings. Other added features are : 1) the gas flush option, which enables the creation of the most optimum gas combination in the package by flushing-out the existing air; and 2) the double heat option with constant heat sealing used for sealing packages made of high barrier films to maintain the package integrity during storage. A typical operation of the machine consists of : (i) creating a vacuum (450 to 680 mm of mercury) in the packages so as to evacuate the existing air, (ii) back-flushing with the required gas combination and (iii) sealing the bag using the double seal option. Using these options, the package may be flushed with different gas combination before sealing, to study change in its respiration rate in this modified gas atmosphere.

4.2 Refrigerated B. O. D. Incubator

This model refrigerated incubator has a two-channel, 7-day programmable temperature control system at which any temperature in the range between -20 to 60°C can be set. It has LED temperature display along with a 4", 7 day recording option. A programmable timer controls the switch back and forth between channel 1 and 2. The unit is equipped with high and low temperature alarms. An internal lighting arrangement is also available. The unit is equipped with auto defrost , hence when the cabinet is run below 0°C for extended periods, cooling coil is cleared of ice by allowing the temperature to rise approximately 4°C above zero. The unit has a temperature control accuracy of (±0.15°C) and a temperature uniformity of (± 0.3°C). It has a inside working volume of 19.7 Ft^3 with four adjustable shelves.

4.3 Chamber for determination of Respiration rate

A chamber was used for studies on the determination of respiration rate of different commodities at laboratory level. It consists of a section of a PVC pipe, 500 mm long and 250 mm in diameter. The two ends are closed with a square lid of clear acrylic, 6mm thick, and held in place with six threaded rods. Neoprene gaskets have been used on both ends to ensure near perfect air-tightness. The silicon membrane is installed on the lid in a window like fashion. The unit has the advantage that it is durable, easy to construct, near perfect airtight, quick and simple opening and closing procedures, possibility of visual assessment of the product during storage studies, low maintenance requirement, and ease of stacking. The small size of chamber facilitates putting it in BOD incubator to study the Respiration Rate at different environmental conditions.

5.0 Application of equipment in research / demonstration

The equipment discussed in section 4 in conjunction with the appropriate methods for analysis will be used in shelf-life studies of respiring project such as Saskatoon berries and alfalfa which is of local interest. Research on non-respiring products such as meat and meat products is also planned.

The equipment has been calibrated and programmed and trial runs were conducted on fruits and vegetables. Due to non-availability of Gas Chromatograph (because of lack of funds) proper analysis of MAP systems could not be conducted. However, a sequence of experiments has been planned in summer' 93 on shelf-life studies on fresh alfalfa chops and Saskatoon berries. To aid in the analysis, the Gas Chromatograph in the Department of Geological Science will be used.

Demonstration of the equipment to local food processors and farming community will be held as per the need and requests. The MAP facilities will be open to interested parties for touring and discussions and consultations are possible.

6.0 Budget

Item	Amount (Can $)
1 Gas flush option for Audion/pac vacuum chamber machine	704.40 (US $ 587*1.2)
2. LDPE bags (for packaging studies)	116.79
3. Labor	200.00
Total	**$1021.19**

7.0 Research Studies Undertaken in the Project

7.1 Determination of respiration rate of alfalfa for further application to modified atmosphere packaging

7.1.1 Summary

Gas chromatographic method was applied to study the respiration rate of fresh alfalfa as a function of time and temperature. Both of these factors were found to significantly influence the respiration rate. The results obtained would be useful in evaluating and optimizing several parameters responsible for extension of its shelf-life, specially while applying the technique of modified atmosphere packaging for this purpose.

7.1.2 Introduction

The storage life of harvested fruits, vegetables and pasture plants, is influenced by their continued respiration and the biochemical activity associated with senescence. Low respiration rate is associated with long storage life. Lowering the temperature, and modifying or controlling the atmosphere surrounding the product, reduces respiration and delays senescence, thus extending shelf-life.

In a gas-tight container, living vegetable material modifies the atmosphere, due to respiratory oxygen uptake and release of carbon dioxide. Consequently, equilibrium concentrations of oxygen and carbon dioxide are established. The actual equilibrium modified atmosphere attained within a package will depend on factors such as temperature, respiration rate, O_2 and CO_2 permeabilities of the packaging materials, fill-weight, free volume in the package, and film surface area. An optimum atmosphere minimizes respiration rate without danger of metabolic damage to the commodity.

Alfalfa are highly perishable and deteriorate rapidly after harvesting. To retard this deterioration, it is necessary that the respiration rate of alfalfa is checked, using some of the preservative techniques which retains its freshness, nutritive value and color characteristics as

well. Modified atmosphere packaging has been reported to have great potential for this purpose. For short-lived and commercially valuable produce like alfalfa, their is an economic incentive to extend shelf-life by using modified atmosphere packaging. Moreover, little information is available regarding the changes in the rate of respiration of pasture plants after they have been harvested and are allowed to dry out as during hay-making or other preservation techniques. Hence, with a view to generate a baseline information for application of modified atmosphere packaging for extension of shelf-life of alfalfa, the present study was conducted to measure the respiration rate of alfalfa. The results obtained are presented in this paper.

7.1.3 Materials and Methods

Alfalfa (variety-Beaver) grown at Agriculture Canada farm, Saskatoon were harvested and placed in air-tight HDPE pails of about 20 liter size. A sample size of 675 g and 630 g were used for storage at 20°C and at 12°C respectively. Samples of alfalfa, in triplicate, were kept in oven at 60°C for 72 hours to determine their moisture content. A relatively larger sample was preferred to obtain a representative sample of alfalfa plant. A rubber septum, for gas sampling, were fitted into the lid. Samples of the inside air in the head space of pails were drawn at frequent intervals using a 1 mL syringe and were injected in the Gas Chromatograph (Make-Fisher Hemilton gas partitioner, model 29) equipped with a thermal conductivity detector with four tungsten filament. The column materials were (6 ft x 0.25 inch aluminum packed with 30% DEHS in 60-80 mesh chromosorb and 6.5 ft x 3/16 inch aluminum packed with 40-60 mesh molecular sieve 13X. The cell temperature was 70°C while column temperature was set at ambient temperature. The carrier gas used was helium at pressure of 40 psi with a flow rate of 50 mL min^{-1}. The GC was coupled to a Hewlett-Packard integrator (Model-3390A) which determined the CO_2 concentration in terms of percent volume of air present in the pail. Before each analysis, the Gas Chromatograph (GC) was calibrated for measurement of CO_2 concentration, using a known standard gas concentration. Each analysis was replicated thrice. The sample pails were kept in refrigerated incubator (Make-Revco) maintained at (12±0.1)°C. The incubator was equilibrated at set temperature for one week before study began. Another pail

was kept at room temperature and the temperature of the room was recorded using a hygrothermograph (Make - Cole Parmer). The room temperature was observed to be (20±1)°C. The values of CO_2 produced were converted to respiration rate in terms of mL of CO_2 kg^{-1} h^{-1} using following formula;

$$RR = \frac{(Va-Va) * (\%\ CO_2 \text{ of sample} - \%\ CO_2 \text{ of ambient}) * 1}{Wa\ (kg) * time\ (hour)}$$

where, RR= respiration rate in mL CO_2 kg^{-1} h^{-1}

Va= volume of air in the pail, liter

Va= volume of alfalfa sample in pail, liter

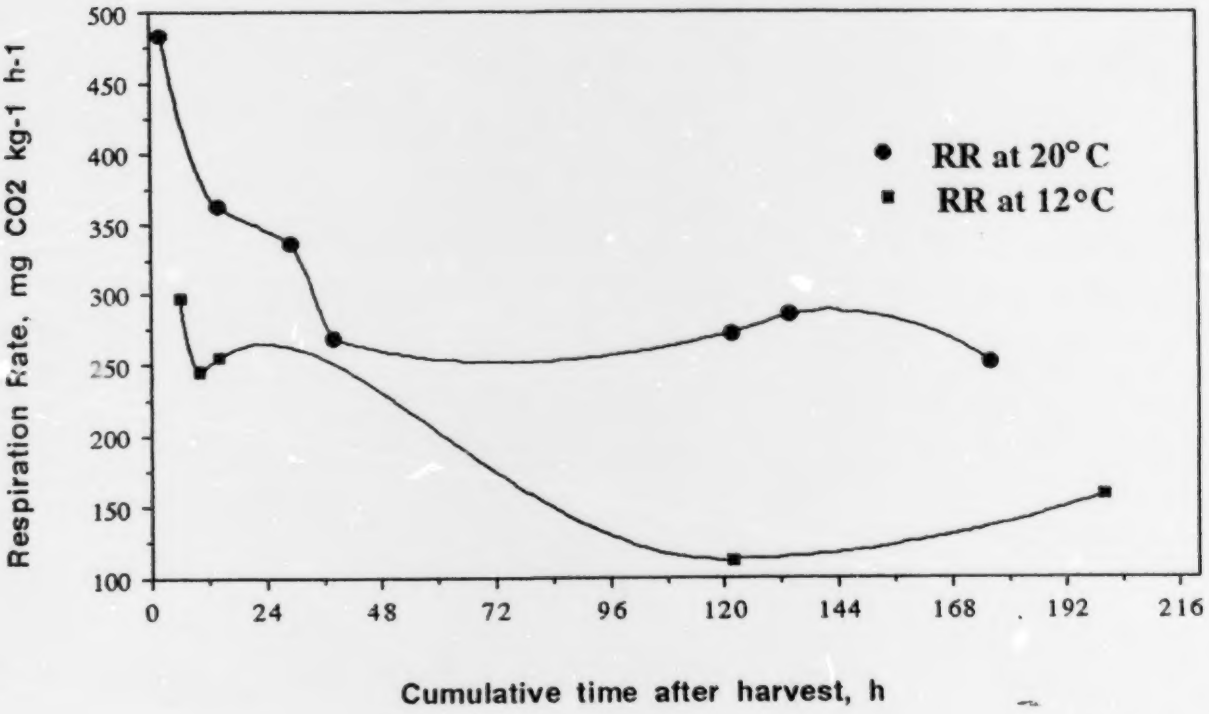
Wa= weight of alfalfa sample, kg

The values of CO_2 obtained were converted in terms of mg of CO_2 kg^{-1} h^{-1} for comparison with the values of respiration rate of different similar commodities.

7.1.4 Results and Discussion

The fresh alfalfa was found to have a moisture content of 78.10% (wet basis). The values of respiration rate of alfalfa obtained were summarized and have been presented in Fig 1. It is observed that the value of respiration rate of alfalfa decreased with time and temperature. Initially, the value was 483.9 mg CO_2 kg^{-1} h^{-1} at 20°C but was found to reduce to a value of 253.05 kg^{-1} h^{-1} after about eight days of storage. It had a decreasing trend except a little increase after 122 and 134 hours. In case of sample stored at 12°C the value of respiration rate was quite low compared with the value of respiration rate at 20°C. It was 298.12 mg CO_2 kg^{-1} h^{-1} and reduced to a value of 158.06 mg CO_2 kg^{-1} h^{-1}. These results can be understood since freshly harvested alfalfa might have been growing actively, hence it exhibited relatively higher rate of respiration. This is also because the respiration gets aggravated due to injury to plant during harvest. However, this activity did not continue in absence of photosynthetic input

Fig 1 Respiration rate of fresh alfalfa (m:c.78%) at two different temperatures



(in an air-tight closed container) and therefore, the respiration rate decreased with time and attained a state of equilibrium. Hence the value of 362.56 mg CO_2 kg^{-1} h^{-1} appears to be more close and stable to actual respiration rate of alfalfa instead of a relatively higher value of 483.9 mg CO_2 kg^{-1} h^{-1}. Similar results were obtained by some previous researchers for the respiration of asparagus at 10°C. The values of respiration rates obtained by him were 196, 108, 83, 70 mg CO_2 kg^{-1} h^{-1} after 1, 2, 3, and 4 days respectively.

The comparison of results of this study on alfalfa with the respiration rate of other similar vegetative commodities indicate (Fig 2) a relatively higher value for alfalfa. This may perhaps be due to the fact that alfalfa used in this study might have been harvested when it was growing actively. This is an important factor which determines the rate of respiration of plant. The stage of growth of crop at the time of harvest significantly affects the respiration rate of the plant.

The results on effect of temperature are also in good agreement with earlier studies (Fig 2). Although the concept that an increase of 10°C doubles the respiration rate, could not be verified because of the fact that in the present study temperatures of 12°C and 20°C were used. The result trend, however, appears to be quite towards that.

7.1.5 Conclusions

The respiration rate of alfalfa was found to be 256 mg CO_2 kg^{-1} h^{-1} and 363 mg CO_2 kg^{-1} h^{-1} at 12°C and at 20°C respectively. The results of present study indicate that with the instrumentation and facilities available an in-depth study on application of modified atmosphere packaging of alfalfa can be initiated.

8.0 References

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